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Age-related morphometric changes of the tidemark in the ovine stifle

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Age-related morphometric changes of the tidemark in the ovine stifle

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Keywords:	sheep, cartilage, knee, osteoarthritis, ageing
Abstract:	<p>Though the ovine stifle is commonly used to study osteoarthritis, there is limited information about the age-related morphometric changes of the tidemark. The objective of this study was to document the number of tidemarks in the stifle of research sheep without clinical signs of osteoarthritis and of various ages (n = 80). Articular cartilage of the medial and lateral tibial condyles and of the medial and lateral femoral condyles was assessed by histology: (1) to count the number of tidemark; and (2) to assess the OARSI (OsteoArthritis Research Society International) score for structural changes of cartilage.</p> <p>The number of tidemarks varied between anatomical regions respectively from 4.2 in the medial femoral condyle to 5.0 in the lateral tibial condyle. The axial part showed a significant higher number of tidemarks than the abaxial part, for all regions except the medial tibial condyle. While the tidemark count strongly correlated to age (Spearman Correlation coefficient=0.70; 95% confidence interval 0.67 to 0.73; $P<0.0001$), the OARSI score was weakly correlated to age in our cohort of sheep (Spearman Correlation coefficient=0.25; 95% confidence interval 0.19 to 0.30; $P<0.0001$). Interestingly, no tidemark was seen in the three animals aged 6 months.</p> <p>Our data indicate that the number of tidemarks increases with age and vary with anatomical region. The regional variation also revealed a higher number of tidemarks in the tibia than in the femur. This could be attributed to the local variation in cartilage response to strain and to the</p>

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	difference in chondrocyte biology and density.

SCHOLARONE™
Manuscripts

Age-related morphometric changes of the tidemark in the ovine stifle.

Running title: Tidemark in the ovine stifle

Fanny Hontoir¹, Romain Pirson¹, Vincent Simon¹, Peter Clegg², Jean-François Nisolle,
Nathalie Kirschvink¹, Jean-Michel E. Vandeweerdt¹

¹ Department of Veterinary Medicine, Integrated Veterinary Research Unit (IVRU) – Namur
Research Institute for Life Sciences (NARILIS), Faculty of Sciences, University of Namur, rue
de Bruxelles, 61, 5000 Namur, Belgium

² Department of Musculoskeletal Biology, Institute of Ageing and Chronic disease, University
of Liverpool, Liverpool L69 3BX, UK

³ Centre Hospitalier Universitaire (CHU) UCL Namur-Mont Godinne, Université Catholique
de Louvain, 5530 Yvoir, Belgium

Corresponding author:

Fanny Hontoir

61, rue de Bruxelles

5000 Namur

Tel: 0032 496 53 51 45

fanny.hontoir@unamur.be

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Summary

Though the ovine stifle is commonly used to study osteoarthritis, there is limited information about the age-related morphometric changes of the tidemark. The objective of this study was to document the number of tidemarks in the stifle of research sheep without clinical signs of osteoarthritis and of various ages (n = 80). Articular cartilage of the medial and lateral tibial condyles and of the medial and lateral femoral condyles was assessed by histology: (1) to count the number of tidemark; and (2) to assess the OARSI (OsteoArthritis Research Society International) score for structural changes of cartilage.

The number of tidemarks varied between anatomical regions respectively from 4.2 in the medial femoral condyle to 5.0 in the lateral tibial condyle. The axial part showed a significant higher number of tidemarks than the abaxial part, for all regions except the medial tibial condyle. While the tidemark count strongly correlated to age (Spearman Correlation coefficient=0.70; 95% confidence interval 0.67 to 0.73; P<0.0001), the OARSI score was weakly correlated to age in our cohort of sheep (Spearman Correlation coefficient=0.25; 95% confidence interval 0.19 to 0.30; P<0.0001). Interestingly, no tidemark was seen in the three animals aged 6 months.

Our data indicate that the number of tidemarks increases with age and vary with anatomical region. The regional variation also revealed a higher number of tidemarks in the tibia than in the femur. This could be attributed to the local variation in cartilage response to strain and to the difference in chondrocyte biology and density.

Key words: sheep – cartilage – stifle – osteoarthritis - ageing

Number of figures in this manuscript: 4

Number of tables in this manuscript: 1

Introduction

Osteoarthritis is a degenerative process of the diarthrodial (synovial) joint characterized by progressive degeneration of the articular cartilage, combined with subchondral bone sclerosis and osteophyte formation, leading to reduced joint function (Grynpas, Albert, Katz, Lieberman, Pritzker, 1991; McIlwraith, 1996, p.34). Histology is considered as a gold standard technique to assess normality of cartilage, disease development (Lahm, Kreuz, Oberst, Haeberstroh, Uhl et al., 2006; Wucherer, Ober, Cozemius, 2012; Zamli, Adams, Tarlton, Sharif, 2013), and efficacy of treatments (Huang, Simonian, Norman, Clark, 2004; Hoeman, Hurtig, Rossomacha, Sun, Chevrier et al., 2005; Zscharnak, Hepp, Richter, Aigner, Schultz et al., 2010) in research studies on osteoarthritis.

Different scoring scales have been described for microscopic assessment of cartilage, based on several histological criteria such as the Mankin score, the “modified Mankin score” (Thomas, Fuller, Whittles, Sharif, 2007; Piskin, Gulbahar, Tomak, Gukman, Hokelek et al., 2007; Daubs, Markel, Manley, 2006), and the ICRS (International Cartilage Repair Society) -II scoring scale (Mainil-Varlet, Van Damme, Nesic, Knutsen, Kandel, Roberts et al., 2010). Species-specific scoring scales have been proposed by the Osteoarthritis Research Society International (OARSI) histopathology initiative to ensure comparison between studies using animal models of osteoarthritis, in mice (Glasson, Chambers, Van Den Berg, Little, 2010), rats (Gerwin, Bendele, Glasson, Carlson, 2010), guinea pigs (Kraus, Huebner, DeGroot, Bendele, 2010), rabbits (Lavery, Girard, Williams, Hunziker, Pritzker, 2010), dogs (Cook, Kuroki, Visco, Pelletier, Schulz et al., 2010), horses (McIlwraith, Frisbie, Kawcak, Fuller, Hurtig et al., 2010), goats and sheep (Little, Smith, Cake, Read, Murphy et al., 2010). For example in sheep, the histopathological assessment includes the following parameters: cartilage structure, percentage of the surface area affected by structural damage, chondrocyte density, cell cloning, interterritorial Toluidine blue staining, and tidemark variations.

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6 79 The tidemark is the limit between the hyaline cartilage and the calcified cartilage (Meachim &
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8 80 Allibone, 1984; Oegema, Carpenter, Hofmeister, Thompson, 1997; Burr, 2004). At
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10 81 microscopy, the tidemark appears as a non-cellular line of about 10 µm strongly stained with
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12 82 hematoxylin-eosin, or toluidine blue (Lyons, Stoddart, McClure, McClure, 2005). A trilaminar
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14 83 organization has been demonstrated by combining different histochemical staining
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16 84 (hematoxylin and eosin, picrosirius red, toluidine blue and safranin O), with a distal lamina (to
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18 85 the side of the non-calcified cartilage), a proximal lamina (to the side of the calcified-cartilage)
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20 86 and a central lamina. The proximal and distal laminae differ in their chemistry and, hence, in
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22 87 their tinctorial properties. It is therefore suggested that the central lamina is actually an
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24 88 artefactual zone due to the interpenetration of colorations of the proximal and the distal laminae
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26 89 (Lyons et al., 2005).
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30 90 The general consensus is that the tidemark is the result of accumulation of non-specific
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32 91 molecules at the interface of calcified and hyaline cartilage caused by discontinuous
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34 92 mineralization (Oegema et al., 1997). The tidemark seems to be derived from apoptotic
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36 93 chondrocytes, and to include several molecules such as phospholipides, alkaline phosphatase,
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38 94 type X collagen, adenosine triphosphatase, deoxyribonucleic acid, lectins, and High Mobility
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40 95 Group Box chromosomal protein 1 (HMGB1) (Lyons et al. 2005; Simkin 2012). Chondrocytes
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42 96 are not present in the tidemark but a few can be partially embedded in its mineralizing side
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44 97 (Lyons et al., 2005).
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51 99 Tidemark alterations, i.e. duplication, advancement and vascular invasion have been associated
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53 100 to disease such as rheumatoid arthritis (Fassbender, Seibel, Hebert, 1992; Suber & Rosen, 2009)
54
55 101 or osteoarthritis (Oettmeier, Abendroth, Oettmeier, 1989; Bonde et al., 2005; Hulth, 1993; Suri,
56
57 102 Gill, Massena de Camin, Wilson, McWilliams et al., 2007; Bullough & Jagannath, 1983;
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Oegema et al., 1997). In the OARSI score, it is observed whether the tidemark is duplicated (score 1) and whether blood vessels from the subchondral bone cross the tidemark to the calcified cartilage (score 2) or to the hyaline cartilage (score 3).

However, multiple tidemarks can be observed in normal joints (Oegema et al., 1997; Oettmeier et al., 1989). The number of tidemarks has been reported to change with ageing in humans, with an average increase from 1.5 to 2.5 in femur and humerus after the age of 60 (Lane & Bullough, 1980). Duplicated tidemarks were visible in mature normal canine femoral articular cartilage (Oegema et al., 1997). In a study on 28 cynomolgus monkeys, as many as ten tidemarks were observed in normal primates over 20 years old while at least two tidemarks were present in all animals (Miller, Novatt, Hamerman, Carlson, 2004). In horses, the number of tidemarks was higher in non-athletic than in racehorses with articular pathology (Muir, Peterson, Sample, Scollay, Markell, 2008). In non-working and working German shepherd dogs, the tidemark duplication in the femur and the tibia has been suggested to be related to ageing (Francuski, Radovanović, Andrić, Krstić, Bogdanović et al., 2014).

Since tidemark duplication and advancement could be observed in diseased but also in healthy animals, it is important to document how tidemark varies with age in a population of research animals. The sheep, in particular, is commonly used as a large animal model for osteoarthritis (Little et al., 2010). In sheep, there is limited information about the variation of the number of tidemarks (Appleyard, Burkhardt, Ghosh, Read, Cake et al., 2003; Frisbie, Cross, McIlwraith, 2006). Most of the sheep used in research are skeletally mature sheep (Huang et al., 2004; Burger, Mueller, Wlodarczyk, Goost, Tolba et al., 2007; Dattena, Pilichi, Rocca, Mara, Casu et al., 2009) aged between 3 and 6 years old (Hoeman et al., 2005).

The objectives of this study were to document the variation of the number of tidemarks of the stifle in a large cohort of sheep without clinical signs of osteoarthritis and of various ages.

Materials and methods

Population

Eighty pairs of hindlimbs were collected, between 2012 and 2018, from crossed Texel ewes, euthanatized for reasons other than hind limb lameness (mastitis, metritis), within six hours of euthanasia. Animals were aged between 6 months and 3 years old (N=28), 4 to 6 years old (N=31) and 7 to 11 year old (N=21). Animals had no clinical signs of osteoarthritis (lameness, articular swelling, and pain at manipulation). They had been used for teaching anatomy and were not euthanized for the purpose of the current study. The experimental protocol (KI 10/148) was approved by the local ethical committee for animal welfare.

Gross anatomy

After soft tissue dissection and joint opening, synovium and articular surfaces were assessed by one investigator in a blinded manner following OARSI recommendations (Little et al., 2010). Synovium was evaluated for macroscopic alterations (normal, slight, mild, moderate, marked and severe): discoloration, vascularity, thickening and synovial proliferation. Macroscopic scores for cartilage damages were: score 0 for intact cartilage surface; score 1 for surface roughening; score 2 for deeper defects (fibrillation, fissures) not involving the subchondral bone; score 3 for erosions down to the subchondral bone (less than 5 mm diameter); score 4 for large erosions down to the subchondral bone (more than 5 mm diameter). Scoring was performed in four areas of interest: the middle part of the medial tibial condyle (or plateau) (MTC), of the medial femoral condyle (MFC), of the lateral tibial condyle (LTC) and of the lateral femoral condyle (LFC) (Figure 1). Joint margins were observed for the presence of osteophytes. Joint surfaces were digitally photographed (Sony Alpha DSLR-A230 digital camera) with standardized lighting conditions for records (two Sony Illustar SM-300 lighting).

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154 *Histology*

155 Four mm-thick osteochondral slabs were collected from the middle part of the medial tibial
156 condyle (or plateau), medial femoral condyle, lateral tibial condyle and lateral femoral condyle
157 (Figure 1). A total of 640 samples (80 sheep x 2 limbs x 4 regions) were collected. After 48-h
158 fixation in 10% (v/v) neutral buffered formalin, samples were transferred to 70% (v/v) ethanol
159 for further processing (Little et al., 2010). They were decalcified in DC3 (non-ionic surfactants,
160 hydrochloric acid, EDTA, VWR International, Leuven, Belgium) for 2 days and embedded in
161 paraffin, and then 4- μ m sections were cut. Sections were deparaffinised with xylene and graded
162 ethanol, and then stained with Toluidine blue.

163 Each slice was examined for cartilage structure and tidemark count. Scoring of cartilage
164 structure followed the OARSI recommendations for histological evaluation of structural
165 changes in ovine articular cartilage (Little et al., 2010). Each region being divided into two
166 subregions (abaxial (Ab) and axial (Ax)), 1280 subregions were assessed (640 regions x 2).
167 Assessments were performed in duplicates by two observers to obtain a mean score. Tidemark
168 counts were obtained by one blinded observer in six equidistant locations per anatomical region.
169 Mean number was calculated and recorded. Sheep, age and limb identities were blinded to
170 histological scorers.

171

172 *Statistical analysis*

173 Statistics were performed with GraphPad Prism 7.03 (GraphPad Software, La Jolla). Statistical
174 significance was set at 0.05. Firstly, the dataset was assessed for normality, skewness and
175 kurtosis. Due to the moderate positive skewness, to kurtosis, and to non-normal distribution of
176 the data, nonparametric statistics were conducted (Pearce & Frisbie, 2010). Wilcoxon matched-

pairs signed rank test and Friedman test were used to compare data from left and right limbs, and to compare data from the different (sub-)regions of each limb. Kruskal-Wallis test followed by a Dunn's multiple comparison test enabled to test difference between age groups for tidemark count and OARSI scoring. Mean tidemark count and mean OARSI scores of both limbs was considered for each sheep. Correlation between age and tidemark number or OARSI scoring of the sheep was assessed using the Spearman's rank order test. Correlation was considered very weak (0.00-0.19), weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79) and very strong (0.80-1.00) depending on the absolute value of the coefficient.

Results

Gross anatomy

Macroscopic assessment of cartilage for the 1280 anatomic areas revealed 911 zones of intact cartilage (71.2%), 315 score-1 lesions (24.6%), 50 score-2 lesions (3.9%) and 4 score-3 lesions (0.3%). Score-2 and -3 erosions were found in 11 of the 80 sheep (13.75%). No score-4 lesion was found. No signs of joint inflammation (effusion, synovitis) and no osteophyte was detected at gross anatomy.

Histology

Thirty slides presented artifacts (folding, shredding, splitting) preventing tidemark count. Thus, 1250 of the 1280 sub-regions were appropriately assessed. There was no significant difference between left and right limbs for tidemark count (P= 0.5898), and for OARSI scores (P = 0.2761).The tidemark count (P<0.0001) showed difference upon (sub-)regions. The axial sub-region had a significant higher number of tidemarks than the abaxial sub-region, for all regions except in the medial tibial condyle (Figure 3). The number

of tidemarks in the four regions was ranked as $MFC < LFC < MTC < LTC$, with an average number of 4.2, 4.5, 4.8 and 5.0, respectively; those differences were statistically significant, except between MFC and LFC.

The OARSI scores significantly differed with (sub-)regions (Figure 4), with the axial sub-regions showing higher scores than abaxial sub-regions ($P < 0.0001$). OARSI scores in the four regions were ranked as $LFC < LTC < MFC < MTC$, with an average score of 2.0, 2.6, 5.0 and 5.3, respectively. The differences were not significant between regions of the same bone.

The three age groups had significant different tidemark count ($P < 0.0001$) and OARSI scores ($P = 0.0197$) (Table 1), with a strong positive correlation between age and the number of tidemarks (Spearman Correlation coefficient = 0.70, 95% confidence interval 0.67 to 0.73; $P < 0.0001$). However, the OARSI score was weakly correlated to age in our cohort of sheep (Spearman Correlation coefficient = 0.25, 95% confidence interval 0.19 to 0.30; $P < 0.0001$). The correlation between OARSI scores and tidemark count was weak as well (Spearman Correlation coefficient = 0.19, 95% confidence interval 0.13 to 0.24; $P < 0.0001$). In the three young animals aged 6 months, no tidemark was visible (Figure 2).

Discussion

In this study, the number of tidemarks increased significantly with age. Interestingly, no tidemark was identified in the three sheep aged 6 months. This is in agreement with reports that calcified cartilage layer does not begin to develop until well into the first year postpartum (Martinelli, Eurell, Les, Fyhrie, Bennett, 2002). In horses, functional adaptation of articular cartilage occurs during maturation (Brama, TeKoppele, Bank, Barneveld, van Weeren, 2002). Cartilage-bone interface is a dynamic area where duplication of the tidemark and thickening of

226 calcified cartilage are due to micro-trauma at the bone cartilage-interface and quick repair
227 process in response to mechanical stresses over time (Burr & Schaffler, 1997).

228 The effect of constraints on tidemark duplication is also illustrated by the variation of number
229 of tidemarks between anatomical regions. Constraints are higher in the medial compartment
230 due to the asymmetry of load bearing and contact area in the stifle (Thomas, Resnick, Alazraki,
231 Daniel, Greenfield, 1975; Baliunas Hurwitz, Ryals, Karrar, Case et al., 2002; Lee-Shee, Dickey,
232 Hurtig, 2007; Taylor, Poepplau, Konig, Ehrig, Zachow, 2011). This is associated with a higher
233 deterioration of cartilage and higher OARSI scores in those anatomical regions, as
234 demonstrated by studies in sheep (Vandeweerd, Hontoir, Kirschvink, Clegg, Nisolle et al.,
235 2013; Hontoir, Clegg, Simon, Kirschvink, Nisolle et al., 2017), and man (Arøen, Løken, Heir,
236 Alvik, Ekeland et al., 2004; Neogi, Felson, Niu, Lynch, Nevitt et al., 2009; Flanigan, Harris,
237 Trinh, Siston, Brophy, 2010). In the current study, OARSI scores were also higher in the medial
238 tibial and femoral condyles than in the lateral tibial and femoral condyles, with the axial side
239 being more affected.

240 In the current study, the number of tidemarks was higher in the tibia than in the femur. A
241 difference in number of tidemarks has also been described in dogs (Francuski et al., 2014). In
242 femoral cartilage, tidemark multiplication was more frequently observed in working dogs than
243 in non-working dogs, whilst in the tibial cartilage it was more frequently observed in non-
244 working dogs. This particularity has not been described elsewhere. However, regional
245 differences of cartilage mechanobiology and cell biology could account for change in tidemark
246 number. Mechanically, the cartilage strain is not homogeneous through the joint after exercise:
247 for example, in human, the cartilage strain (percentage of thickness change) is higher in the
248 tibia (30%) compared to the femur (20%) after a 30-minutes jogging (Moscher, Smith, Collins,
249 Liu, Hancy et al., 2005; Sanchez-Adams, Leddy, McNulty, O'Connor, Guilak, 2014). Moreover,
250 the cartilage response to loading is different for tibial and femoral cartilage. *In vivo* assessment

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3 251 of cartilage response to load has been performed in human using compositional imaging, this
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5 252 technique revealed that tibial cartilage showed an homogeneous response for deep and
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7 253 superficial layers, whilst the femur showed an opposite response for both layers, suggesting a
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9 254 transport of water to the deep zone of cartilage in the femur, in opposition to the general squeeze
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11 255 of water of both tibial layers (Souza, Kumar, Calixto, Singh, Schooler et al., 2014).
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13 256 Biologically, tibial and femoral cartilage shows different pattern, with higher
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15 257 glycosaminoglycans and collagen content, higher chondrocyte density and proliferation rate in
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17 258 the femur than in the tibia (Stenhamre, Slynarski, Petrén, Tallheden, Lindahl, 2008). It should
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19 259 be reminded here that chondrocyte reaction to mechanical load varies from enhanced matrix
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21 260 synthesis (anabolism) to catabolism, apoptosis and necrosis depending on the frequency, the
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23 261 amplitude, or the strain-scheme for example (Sanchez-Adams et al., 2014; Bleuel, Zacke,
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25 262 Brüggemann, Niehoff, 2015; Iijima, Ito, Nagai, Tajino, Yamaguchi et al., 2017). As the
26
27 263 tidemark originates from the chondrocytes activity (Havelka, Horn, Spohrová, Valouch, 1984)
28
29 264 and apoptosis (Simkin, 2012), the higher number of tidemarks in the tibia could be explained
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31 265 by the combination of higher strain and lower cell yield in the tibia compared to the femur.
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40 267 The correlation between the number of tidemarks and the OARSI score was weak in our sheep
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42 268 population. In a recent research study in man, the tidemark count poorly and non-significantly
43
44 269 correlated to the human OARSI scores in the middle part of 42 lateral tibial condyles, with
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46 270 OARSI scores ranging from 0 (normal) to 4 (superficial delamination to mid-zone erosion).
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48 271 (Deng, Wang, Yin, Chen, Guo et al., 2016). These results support the idea, also proposed by
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50 272 other authors (Lane & Bullough, 1980; Bonde et al., 2005; Oegema et al., 1997; Muir et al.,
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52 273 2008; Francuski et al., 2014), that tidemark multiplication is not a unique feature of
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54 274 osteoarthritis and can be found in normal animals. OARSI scores in the current study were low.
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3 275 In addition, we found no osteophytes, a feature of osteoarthritis (Little et al., 2010; Cake, Read,
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5 276 Corfield, Daniel, Burkhardt et al., 2013).
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10 278 Since there was no osteoarthritic sheep in the current research population, it is not possible to
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12 279 infer on the association between OA and the number of tidemarks. The use of the sheep as an
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14 280 animal model for osteoarthritis requires the surgical induction of the disease to ensure the
15
16 281 development of moderate to severe cartilage damages (Little et al., 2010). For example, in a
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18 282 lateral meniscectomy model, average OARSI scores can reach up to 16 +/-3 for cartilage (with
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20 283 erosion of cartilage and loss of proteoglycans to the mid/deep zone), associated to moderate
21
22 284 synovitis and osteophytes in the lateral femoral and tibial condyles (Gelse, Körber, Schöne,
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24 285 Raum, Koch, 2017). Obviously such cases were not identified in the current population.
25
26 286 One could argue that the decalcification process is a limitation of the current study and would
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28 287 impair assessment of the tidemark. The tidemark is basically seen as the limit between the
29
30 288 calcified cartilage and the hyaline cartilage (Meachim & Allibone, 1984; Oegema et al., 1997;
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32 289 Burr, 2004; Lyons et al., 2005). However, the tidemark is not only featured by presence of
33
34 290 calcium deposition; it contains multiple molecules (phospholipids, alkaline phosphatase,
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36 291 adenosine triphosphatase, DNA, lectins) revealed by a wide range of histologic stains
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38 292 (Dmitrovsky, Lane and Bullough, 1978; Havelka et al., 1984; Oettmeir et al., 1989; Lyons et
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40 293 al., 2005). Furthermore, we have purposely conducted the study according to the OARSI
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42 294 recommendation for assessment of cartilage and osteochondral junction in ovine, i.e. with a
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44 295 decalcification step during the histological processing of osteochondral samples (Little et al.,
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46 296 2010). Another limitation is the lack of one-year old sheep to determine the apparition of the
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48 297 first tidemark. Those animals are not frequently available for research since they are young
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50 298 skeletally mature animal at the beginning of their reproductive career, and therefore not likely
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52 299 to be reformed.
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301 Conclusion

302 Documentation of animal models is a concern in research and should be pursued to ensure
303 accurate evaluation of the model and of the tested hypothesis. In the current study, we
304 demonstrated that the multiplication of the tidemark is associated to ageing in the stifles of our
305 sheep population aged between 6 months and 11 years old, without clinical signs of
306 osteoarthritis. The tidemark count was weakly correlated to OARSI scores, confirming that
307 tidemark count is not a feature of osteoarthritis. This might have implications in the
308 interpretation of the OARSI histological score in sheep. Indeed, ageing seems to be more
309 relevant to tidemark count than osteoarthritis progression in the sheep, as well as in man and
310 dogs.

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315
316 **Conflict of interest statement**

317 None of the authors of this paper has a financial or personal relationship with people or
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323
324 **References**

325 Appleyard, R.C., Burkhardt, D., Ghosh, P., Read, R., Cake, M., Swain, M.V., & Murrell, G.A.
326 (2003). Topographical analysis of the structural, biochemical and dynamic biomechanical
327 properties of cartilage in an ovine model of osteoarthritis. *Osteoarthritis and Cartilage*, 11, 65-
328 77. <https://doi.org/10.1053/joca.2002.0867>.
329 Arøen, A., Løken, S., Heir, S., Alvik, E., Ekeland, A., Granlund, O.G., & Engebretsen, L.
330 (2004). Articular cartilage lesions in 993 consecutive knee arthroscopies. *American Journal of*
331 *Sports Medicine*, 32, 211–215. <https://doi.org/10.1177/0363546503259345>.
332 Baliunas, A.J., Hurwitz, D.E., Ryals, A.B., Karrar, A., Case, J.P., Block, J.A., & Andriacchi,
333 T.P. (2002). Increased knee joint loads during walking are present in subjects with knee
334 osteoarthritis. *Osteoarthritis and Cartilage*, 10, 573-579. doi:10.1053/joca.2002.0797.

- 335 Bleuel, J., Zaucke, F., Brüggemann, GP., & Niehoff, A. (2015). Effects of cyclic tensile strain
336 on chondrocyte metabolism: a systematic review. *PLoS One*, 10, e0119816. doi:
337 10.1371/journal.pone.0119816.
- 338 Bonde, H.V., Talman, M.L.M., & Kofoed, H. (2005). The area of the tidemark in osteoarthritis:
339 a three-dimensional stereological study in 21 patients. *Acta pathologica, microbiologica et*
340 *immunologica Scandinavica*, 113, 349-352. [https://doi.org/10.1111/j.1600-](https://doi.org/10.1111/j.1600-0463.2005.apm_113506.x)
341 [0463.2005.apm_113506.x](https://doi.org/10.1111/j.1600-0463.2005.apm_113506.x)
- 342 Brama, P.A., TeKoppele, J.M., Bank, R.A., Barneveld, A., & van Weeren, P.R. (2002).
343 Development of biochemical heterogeneity of articular cartilage: influences of age and exercise.
344 *Equine Veterinary Journal*, 34, 265-269. <https://doi.org/10.2746/042516402776186146>.
- 345 Bullough, P.G., & Jagannath, A. (1983). The morphology of the calcification front in articular
346 cartilage. *Journal of Bone and Joint Surgery*, 65B, 72-78. doi: 10.1302/0301-
347 620X.65B1.6337169.
- 348 Burger, C., Mueller, M., Wlodarczyk, P., Goost, H., Tolba, R.H., Rangger, C., Kabir, K., &
349 Weber, O. (2007). The sheep as a knee osteoarthritis model: early cartilage changes after
350 meniscus injury and repair. *Laboratory animals*, 41, 420-431. doi:
351 10.1258/002367707782314265.
- 352 Burr, D.B., 2004. Anatomy and physiology of the mineralized tissues: role in the pathogenesis
353 of osteoarthrosis. *Osteoarthritis and Cartilage*, 12, S20-S30.
354 <https://doi.org/10.1016/j.joca.2003.09.016>.
- 355 Burr, D.B., & Schaffler, M.B. (1997). The involvement of subchondral mineralized tissues in
356 osteoarthrosis: quantitative microscopic evidence. *Microscopic research techniques*, 37, 343-
357 357. [https://doi.org/10.1002/\(SICI\)1097-0029\(19970515\)37:4<343::AID-JEMT9>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<343::AID-JEMT9>3.0.CO;2-L)
- 358 Cake, M.A., Read, R.A., Corfield, G., Daniel, A., Burkhardt, D., Smith, M.M., & Little, C.B.
359 (2013). Comparison of gait and pathology outcomes of three meniscal procedures for induction

- 360 of knee osteoarthritis in sheep. *Osteoarthritis and Cartilage*, 21, 226-36. doi:
361 10.1016/j.joca.2012.10.001.
- 362 Clark, J.M., & Huber, J.D. (1990). The structure of the human subchondral plate. *Journal of*
363 *Bone and Joint Surgery Britain*, 72, 866-873. doi: 10.1302/0301-620X.72B5.2211774.
- 364 Cook, J.L., Kuroki, K., Visco, D., Pelletier, J.-P., Schulz, L., & Lajeber, F.P.J.G. (2010). The
365 OARSI histopathology initiative - recommendations for histological assessments of
366 osteoarthritis in the dog. *Osteoarthritis and Cartilage*, 18: S66-S79. doi:
367 10.1016/j.joca.2010.04.017.
- 368 Dattena, M., Pilichi, S., Rocca, S., Mara, L., Casu, S., Masala, G., Manunta, L., Manunta, A.,
369 Passino, E.S., Pool, R.R., & Cappai, P. (2009). Sheep embryonic stem-like cells transplanted
370 in full-thickness cartilage defects. *Journal of tissue engineering and regenerative medicine*, 3,
371 175-187. doi: 10.1002/term.151.
- 372 Daubs, B.M., Markel, M.D., & Manley, P.A. (2006). Histomorphometric analysis of articular
373 cartilage, zone of calcified cartilage, and subchondral bone plate in femoral heads from
374 clinically normal dogs and dogs with moderate or severe osteoarthritis. *American Journal of*
375 *Veterinary Research*, 67, 1719-1724. <https://doi.org/10.2460/ajvr.67.10.1719>.
- 376 Deng, B., Wang, F., Yin, L., Chen, C., Guo, L., Chen, H., Gong, X., Li, Y., & Yang, L. (2016).
377 Quantitative study on morphology of calcified cartilage zone in OARSI 0-4 cartilage from
378 osteoarthritic knees. *Current Research in Translational Medicine*, 64, 149-154. doi:
379 10.1016/j.retram.2016.01.009.
- 380 Dmitrovsky, E., Lane, L.B., & Bullough, P.G. (1978). The characterization of the tidemark in
381 human articular cartilage. *Metabolic Bone Disease and Related Research*, 1, 115-118.
382 [https://doi.org/10.1016/0221-8747\(78\)90047-4](https://doi.org/10.1016/0221-8747(78)90047-4).

- 383 Fassbender, H.G., Seibel, M., & Hebert, T. (1992). Pathways of destruction in metacarpal and
384 metatarsal joints of patients with rheumatoid arthritis. *Scandinavian Journal of Rheumatology*,
385 21, 10-16. <https://doi.org/10.3109/03009749209095055>.
- 386 Flanigan, D.C., Harris, J.D., Trinh, T.Q., Siston, R.A., & Brophy, R.H. (2010). Prevalence of
387 chondral defects in athletes' knees: a systematic review. *Medicine and science in sports and*
388 *exercise*, 42, 1795-801. doi: 10.1249/MSS.0b013e3181d9eea0.
- 389 Francuski, J.V., Radovanović, A., Andrić, N., Krstić, V., Bogdanović, D., Hadžić, V.,
390 Todorović, V., Lazarević Macanović, M., Sourice Petit, S., Beck-Cormier, S., Guicheux, J.,
391 Gauthier, O., & Kovacević Filipović, M. (2014). Age-related changes in the articular cartilage
392 of the stifle joint in non-working and working German shepherd dogs. *Journal of comparative*
393 *pathology*, 151, 363-374. doi: 10.1016/j.jcpa.2014.09.002.
- 394 Frisbie, D.D., Cross, M.W., & McIlwraith, C.W. (2006). A comparative study of articular
395 cartilage thickness in the stifle of animal species used in human pre-clinical studies compared
396 to articular cartilage thickness in the human knee. *Veterinary and Comparative Orthopaedics*
397 *and Traumatology*, 19, 142-146. doi:10.1055/s-0038-1632990.
- 398 Gelse, K., Körber, L., Schöne, M., Raum, K., Koch, P., Pachowsky, M., Welsch, G., & Breiter,
399 R. (2017). Transplantation of Chemically Processed Decellularized Meniscal Allografts.
400 *Cartilage*, 8, 180-190. doi: 10.1177/1947603516646161.
- 401 Gerwin, N., Bendele, A.M., Glasson, S., & Carlson, C.S. (2010). The OARSI histopathology
402 initiative - recommendations for histological assessments of osteoarthritis in the rat.
403 *Osteoarthritis and Cartilage*, 18: S24-S34. doi: 10.1016/j.joca.2010.05.030.
- 404 Glasson, S.S., Chambers, M.G., Van Den Berg, W.B., & Little, C.B. (2010). The OARSI
405 histopathology initiative - recommendations for histological assessments of osteoarthritis in the
406 mouse. *Osteoarthritis and Cartilage*, 18: S17-S23. doi: 10.1016/j.joca.2010.05.025.

- 407 Gryn timer, M., Albert, B., Katz, I., Lieberman, I., & Pritzker, K.P.H. (1991). Subchondral bone
408 in osteoarthritis. *Calcified Tissue International*, 49, 20–26. Doi: 10.1007/BF02555898.
- 409 Havelka, S., Horn, V., Spohrová, D., & Valouch, P. (1984). The calcified-non calcified cartilage
410 interface: the tidemark. *Acta Biologica Hungary*, 35, 271-279.
- 411 Hoeman, C.D., Hurtig, M., Rossomacha, E., Sun, J., Chevrier, A., Shive, M.S., & Buschmann,
412 M.D. (2005). Chitosan-Glycerol Phosphate/Blood Implants improve Hyaline Cartilage Repair
413 in Ovine Microfracture Defects. *The Journal of Bone And Joint Surgery*, 87, 2671-2686.
414 doi:10.2106/JBJS.D.02536.
- 415 Hoemann, C., Kandel, R., Roberts, S., Saris, D.B.F., Creemers, L., Mainil-Varlet, P., Méthot,
416 S., Hollander, A.P., & Buschmann, M.D. (2011). International Cartilage Repair Society (ICRS)
417 Recommended Guidelines for Histological Endpoints for Cartilage Repair Studies in Animal
418 Models and Clinical Trials. *Cartilage*, 2, 153– 172. doi: 10.1177/1947603510397535.
- 419 Hontoir, F., Clegg, P., Simon, V., Kirschvink, N., Nisolle, J.-F., & Vandeweerdt, J.-M. (2017).
420 Accuracy of computed tomographic arthrography for assessment of articular cartilage defects
421 in the ovine stifle. *Veterinary Radiology and Ultrasound*, 58, 512-523. doi: 10.1111/vru.12504.
- 422 Huang, F.S., Simonian, P.T., Norman, A.G., & Clark, J.M. (2004). Effects of small
423 incongruities in a sheep model of osteochondral autografting. *The American Journal of sports*
424 *medicine*, 32, 1842-1848. <https://doi.org/10.1177/0363546504264895>.
- 425 Hulth, A. (1993). Does osteoarthritis depend on growth of the mineralized layer of cartilage?
426 *Clinic Orthopaedics Related Research*, 287, 19–24. doi: 10.1097/00003086-199302000-00004.
- 427 Iijima, H., Ito, A., Nagai, M., Tajino, J., Yamaguchi, S., Kiyan, W., Nakahata, A., Zhang, J.,
428 Wang, T., Aoyama, T., Nishitani, K., & Kuroki, H. (2017). Physiological exercise loading
429 suppresses post-traumatic osteoarthritis progression via an increase in bone morphogenetic
430 proteins expression in an experimental rat knee model. *Osteoarthritis and Cartilage*, 25, 964-
431 975. doi: 10.1016/j.joca.2016.12.008.

- 432 Jeffery, A.K., Blunn, G.W., Archer, C.W., & Bentley, G. (1991). Three-dimensional collagen
433 architecture in bovine articular cartilage. *Journal of Bone and Joint Surgery*, 73, 795-801.
434 <https://doi.org/10.1016/j.joca.2017.02.673>.
- 435 Kraus, V.B., Huebner, J.L., DeGroot, J., & Bendele, A. (2010). The OARSI histopathology
436 initiative - recommendations for histological assessments of osteoarthritis in the guinea pig.
437 *Osteoarthritis and Cartilage*, 18, S35-S52. <https://doi.org/10.1016/j.joca.2010.04.015>.
- 438 Lahm, A., Kreuz, P., Oberst, M., Haeberstroh, J., Uhl, M., & Maier, D. (2006). Subchondral
439 and trabecular bone remodelling in canine experimental model of osteoarthritis. *Archives of*
440 *Otrthopaedic and Trauma Surgery*, 126, 582-587. doi: 10.1007/s00402-005-0077-2.
- 441 Lane, L.B., & Bullough, P.G., (1980). Age-related changes in the thickness of the calcified
442 cartilage and the number of tidemarks in adult human articular cartilage. *The journal of bone*
443 *and joint surgery*, 62, 372–375. doi: 10.1302/0301-620X.62B3.7410471.
- 444 Laverty, S., Girard, C.A., Williams, J.M., Hunziker, E.B., & Pritzker, K.P.H. (2010). The
445 OARSI histopathology initiative - recommendations for histological assessments of
446 osteoarthritis in the rabbit. *Osteoarthritis and Cartilage*, 18, S53-S65. doi:
447 10.1016/j.joca.2010.05.029.
- 448 Lee-Shee, N.K., Dickey, J.P., & Hurtig, M.B. (2007). Contact mechanics of the ovine stifle
449 during simulated early stance in gait. An *in vitro* study using robotics. *Veterinary and*
450 *comparative orthopaedics and traumatology*, 20, 70-72. doi: 10.1055/s-0037-1616591.
- 451 Little, C.B., Smith, M.M., Cake, M.A., Read, R.A., Murphy, M.J., & Barry, F.P. (2010). The
452 OARSI histopathology initiative - recommendations for histological assessments of
453 osteoarthritis in sheep and goats. *Osteoarthritis and Cartilage*, 18, 80-92.
454 <http://dx.doi.org/10.1016/j.joca.2010.04.016>.
- 455 Lyons, T.J., Stoddart, R.W., McClure, S.F., & McClure, J. (2005). The tidemark of the chondro-
456 osseous junction of the normal human knee joint. *Journal of molecular histology*, 36, 207–215.
457 <https://doi.org/10.1007/s10735-005-3283-x>.

- 458 Mainil-Varlet, P., Van Damme, B., Nesic, D., Knutsen, G., Kandel, R., & Roberts, S. (2010).
459 A new histology scoring system for the assessment of the quality of human cartilage repair:
460 ICRS II. *American Journal of Sports Medicine*, 38, 880-890. doi: 10.1177/0363546509359068.
461 Martinelli, M.J., Eurell, J., Les, C.M., Fyhrie, D., & Bennett, D. (2002). Age-related
462 morphometry of equine calcified cartilage. *Equine Veterinary Journal*, 34, 274-278.
463 <https://doi.org/10.2746/042516402776186100>.
464 McIlwraith, C.W. (1996). *Joint Disease in the Horse*. Philadelphia, PA: Saunders.
465 McIlwraith, C.W., Frisbie, D.D., Kawcak, C.E., Fuller, C.J., Hurtig, M., & Cruz, A. (2010).
466 The OARSI histopathology initiative - recommendations for histological assessments of
467 osteoarthritis in the horse. *Osteoarthritis and Cartilage*, 18, S93-S105.
468 <https://doi.org/10.1016/j.joca.2010.05.031>.
469 Meachim, G., & Allibone, R. (1984). Topographical variation in the calcified zone of upper
470 femoral articular cartilage. *Journal of Anatomy*, 139, 341-352.
471 Miller, L.M., Novatt, J.T., Hamerman, D., & Carlson, C.S. (2004). Alterations in mineral
472 composition observed in osteoarthritic joints cynomolgus monkeys. *Bone*, 35, 498-506.
473 <https://doi.org/10.1016/j.bone.2004.03.034>.
474 Mosher, T.J., Smith, H.E., Collins, C., Liu, Y., Hancy, J., Dardzinski, B.J., & Smith, M.B.
475 (2005). Change in knee cartilage T2 at MR imaging after running: a feasibility study.
476 *Radiology*, 234, 245-249. <https://doi.org/10.1148/radiol.2341040041>.
477 Muir, P., Peterson, A.L., Sample, S.J., Scollay, S.C., Markell, M.D., & Kalscheur, V.L. (2008).
478 Exercise-induced metacarpophalangeal joint adaptation in the Thoroughbred racehorse.
479 *Journal of anatomy*, 213, 706-717. doi: 10.1111/j.1469-7580.2008.00996.x.
480 Neogi, T., Felson, D., Niu, J., Lynch, J., Nevitt, M., Guermazi, A., Roemer, F., Lewis, C.E.,
481 Wallace, B., & Zhang, Y. (2009). Cartilage loss occurs in the same subregions as subchondral

- bone attrition: a within-knee subregion-matched approach from the multicentre osteoarthritis study. *Arthritis and rheumatism*, 61, 1539-1544. doi: 10.1002/art.24824.
- Oegema, T.R., Carpenter, R.J., Hofmeister, F., & Thompson, R.C. (1997). The interaction of the zone of calcified cartilage and subchondral bone in osteoarthritis. *Microscopy research and technique*, 37, 324–332. [https://doi.org/10.1002/\(SICI\)1097-0029\(19970515\)37:4<324::AID-JEMT7>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<324::AID-JEMT7>3.0.CO;2-K)
- Oettmeier, R., Abendroth, K., & Oettmeier, S. (1989). Analyses of the tidemark on human femoral heads. II. Tidemark changes in osteoarthrosis: a histological and histomorphometric study in non-decalcified preparations. *Acta morphologica Hungarica*, 37, 169-180.
- Pearce, G.L., & Frisbie, D.D. (2010). Statistical evaluation of biomedical studies. *Osteoarthritis and Cartilage* 18, S117-122. doi: 10.1016/j.joca.2010.04.014.
- Piskin, A., Gulbahar, M.Y., Tomak, Y., Gulman, B., Hokelek, M., Kerimoglu, S. Koksall, B., Alic, T., & Kabak, Y.B. (2007). Osteoarthritis models after anterior cruciate ligament resection and medial meniscectomy in rats. A histological and immunohistochemical study. *Saudi Medical Journal*, 28, 1796–1802.
- Sanchez-Adams, J., Leddy, H.A., McNulty, A.L., O'Connor, C.J., & Guilak, F. (2014). The mechanobiology of articular cartilage: bearing the burden of osteoarthritis. *Current Rheumatology Reports*, 16, 451. doi: 10.1007/s11926-014-0451-6. doi: 10.1007/s11926-014-0451-6.
- Simkin, P.A. (2012). Consider the tidemark. *The Journal of Rheumatology*, 39, 890-892. doi: 10.3899/jrheum.110942.
- Souza, R.B., Kumar, D., Calixto, N., Singh, J., Schooler, J., Subburaj, K., Li, X., Link, T.M., & Majumdar, S. (2014). Response of knee cartilage T1rho and T2 relaxation times to *in vivo* mechanical loading in individuals with and without knee osteoarthritis. *Osteoarthritis and Cartilage*, 22, 1367-1376. doi: 10.1016/j.joca.2014.04.017.

- 507 Stenhamre, H., Slynarski, K., Petrén, C., Tallheden, T., & Lindahl, A. (2008). Topographic
508 variation in redifferentiation capacity of chondrocytes in the adult human knee joint.
509 *Osteoarthritis and Cartilage*, 16, 1356-1362. doi: 10.1016/j.joca.2008.03.025.
- 510 Suber, T., & Rosen, A. (2009). Apoptotic cell blebs: repositories of autoantigens and
511 contributors to immune context. *Arthritis and Rheumatism*, 60, 2216-2219. doi:
512 10.1002/art.24715.
- 513 Suri, S., Gill, S.E., Massena de Camin, S., Wilson, D., McWilliams, D.F., & Walsh, D.A.
514 (2007). Neurovascular invasion at the osteochondral junction and in osteophytes in
515 osteoarthritis. *Annals of Rheumatic Diseases*, 66, 1423-1428. doi: 10.1136/ard.2006.063354
- 516 Taylor, W.R., Poeppelau, B.M., Konig, C., Ehrig, R.M., Zachow, S., Duda, G.N., & Heller, M.O.
517 (2011). The medial-lateral force distribution in the ovine stifle joint during walking. *Journal of*
518 *Orthopaedic Research*, 29, 567-571. doi: 10.1002/jor.21254.
- 519 Thomas, C.M., Fuller, C.J., Whittles, C.E., & Sharif, M. (2007). Chondrocyte death by
520 apoptosis is associated with cartilage matrix degradation. *Osteoarthritis and Cartilage*, 15, 27-
521 34. <https://doi.org/10.1016/j.joca.2006.06.012>.
- 522 Thomas, R.H., Resnick, D., Alazraki, N.P., Daniel, D., & Greenfield, R. (1975). Compartmental
523 evaluation of osteoarthritis of the knee: a comparative study of available diagnostic modalities.
524 *Radiology*, 116, 585-94. <https://doi.org/10.1148/116.3.585>.
- 525 Vandeweerdt, J.M., Hontoir, F., Kirschvink, N., Clegg, P., Nisolle, J.F., Antoine, N., & Gustin,
526 P. (2013). Prevalence of Naturally Occurring Cartilage Defects in the Ovine Knee.
527 *Osteoarthritis and Cartilage*, 21, 1125-1131. doi: 10.1016/j.joca.2013.05.006.
- 528 Wucherer, K.L., Ober, C.P., & Conzemius, M.G. (2012). The use of delayed gadolinium
529 enhanced magnetic resonance imaging of cartilage and T2 mapping to evaluate articular
530 cartilage in the normal canine elbow. *Veterinary Radiology and Ultrasound*, 53, 57-63. doi:
531 10.1111/j.1740-8261.2011.01867.x.

- 1
2
3 532 Zamli, Z., Adams, M.A., Tarlton, J.F., & Sharif, M. (2013). Increased Chondrocyte Apoptosis
4
5 533 Is Associated with Progression of Osteoarthritis in Spontaneous Guinea Pig Models of the
6
7 534 Disease. *Internatinal Journal of Molecular Sciences*, 14, 17729-17743. doi:
8
9 535 10.3390/ijms140917729.
10
11
12 536 Zscharnak, M., Hepp, P., Richter, R., Aigner, T., Schultz, R., Somerson, J., Josten, C., Bader,
13
14 537 A., & Marquass, B. (2010). Repair of chronic osteochondral defects using predifferentiated
15
16 538 mesenchymal stem cells in an ovine model. *American Journal of Sports Medicine*, 38, 1857-
17
18 539 1869. doi: 10.1177/0363546510365296.
19
20
21
22
23
24
25
26
27
28
29
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Table 1: Tidemark count and OARSI score values (median and interquartile range) for the three age groups.

	6 months to 3 years old (N = 28)	4 to 6 years old (N = 31)	7 to 11 years old (N = 21)
Tidemark count			
Median	2.67	4.33	6.67
Range	(1.33 – 4.00)	(3.33 – 5.50)	(5.30 – 8.08)
OARSI Scores			
Median	1.50	2.00	3.00
Range	(1.00 – 3.00)	(1.00 – 5.00)	(1.00 – 7.00)

N= number of sheep. Mean tidemark count and OARSI scoring of both limbs were considered for each sheep.

The tidemark count ($P<0.0001$) and the OARSI scores ($P=0.0197$) differed significantly between groups.

Figure legends

Figure 1. Sampling sites in the middle third of the medial tibial condyle (MTC), medial femoral condyle (MFC), lateral tibial condyle (LTC) and lateral femoral condyle (LFC). Tibial slabs were centered on the intercondylar eminence (black lines). Femoral slabs were obtained in the centre of the middle third of the circumference of the condyle (black lines and dotted black box). White rectangles illustrate the histological slices that were obtained, each abaxial (Ab) and axial (Ax) part being assessed separately at microscopy. White arrows highlight cartilage

Figure 2. The osteochondral junction at histology.

A. The white line indicates non-calcified hyaline cartilage (HC); the black line is the calcified cartilage (CC).

B. White arrows indicate tidemarks.

C. Histological slide showing the absence of tidemark in a sample of cartilage of the medial femoral condyle in a 6 months old sheep.

Figure 3: Number of tidemarks in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P < 0.05$) is reached for the difference between the axial and the abaxial part of the region.

MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.

Figure 4: OARSI scores in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P < 0.05$) is reached for the difference between the axial and the abaxial part of the region.

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572 MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral
573 femoral condyle, respectively.

For Review Only

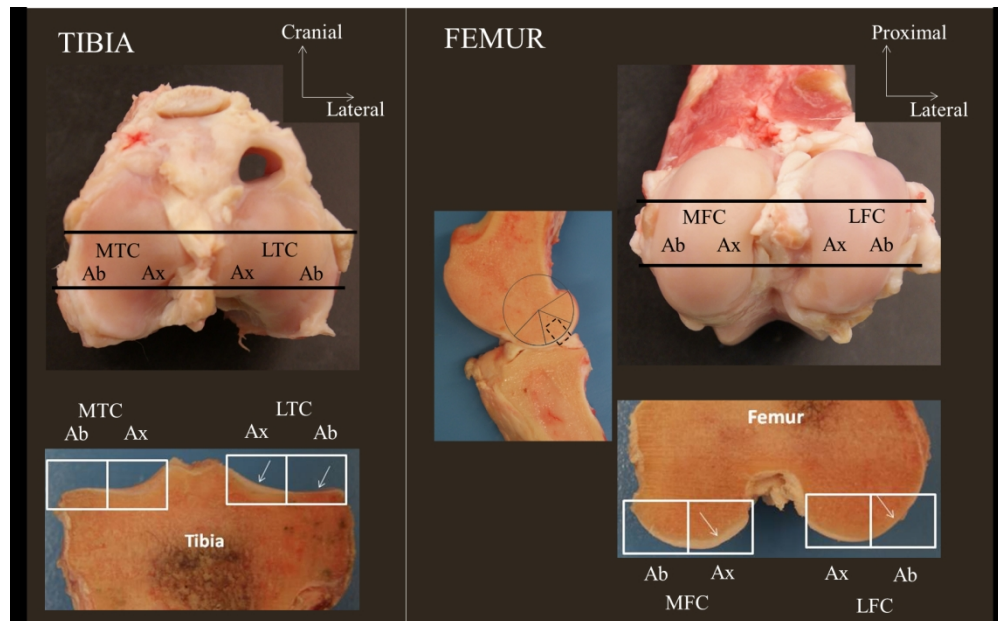


Figure 1. Sampling sites in the middle third of the medial tibial condyle (MTC), medial femoral condyle (MFC), lateral tibial condyle (LTC) and lateral femoral condyle (LFC). Tibial slabs were centered on the intercondylar eminence (black lines). Femoral slabs were obtained in the centre of the middle third of the circumference of the condyle (black lines and dotted black box). White rectangles illustrate the histological slices that were obtained, each abaxial (Ab) and axial (Ax) part being assessed separately at microscopy. White arrows highlight cartilage.

155x96mm (300 x 300 DPI)

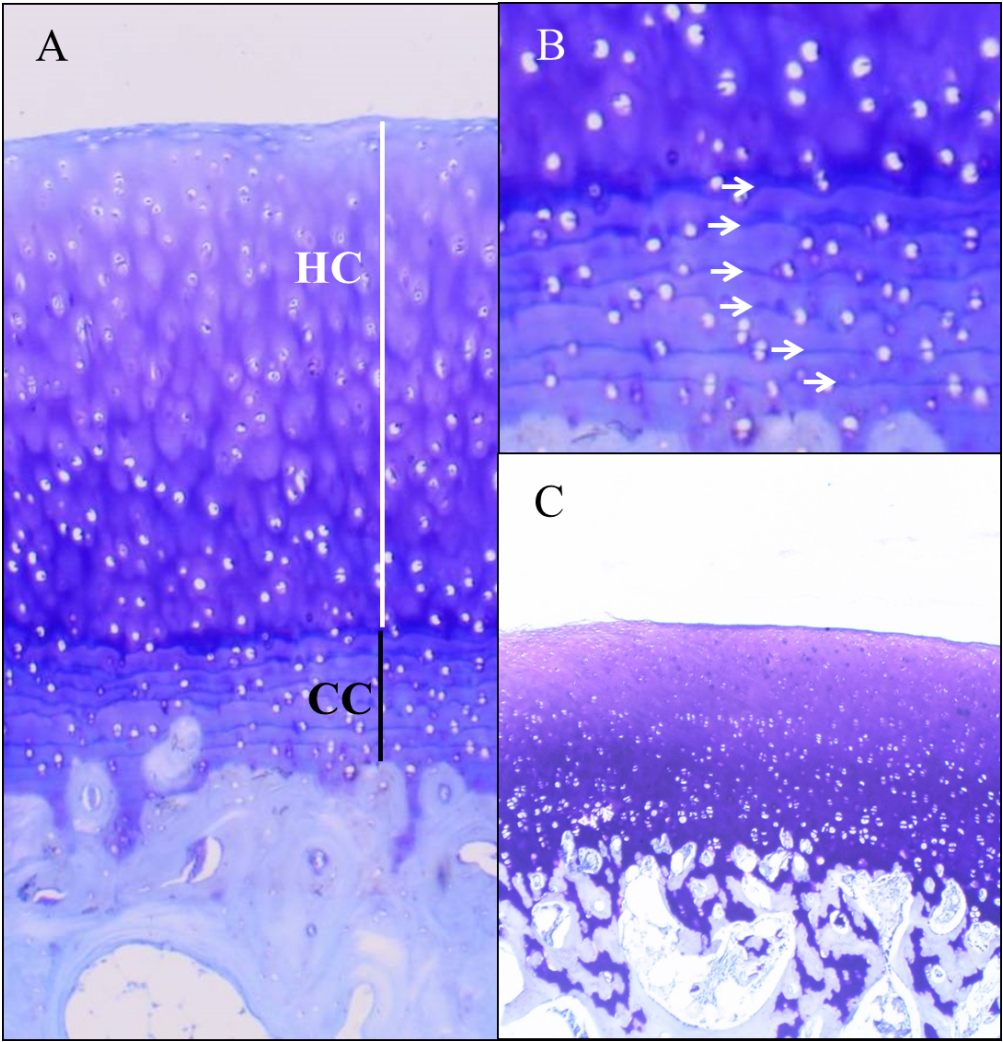


Figure 2. The osteochondral junction at histology.
A. The white line indicates non-calcified hyaline cartilage (HC); the black line is the calcified cartilage (CC).
B. White arrows indicate tidemarks.
C. Histological slide showing the absence of tidemark in a sample of cartilage of the medial femoral condyle in a 6 months old sheep.

92x95mm (300 x 300 DPI)

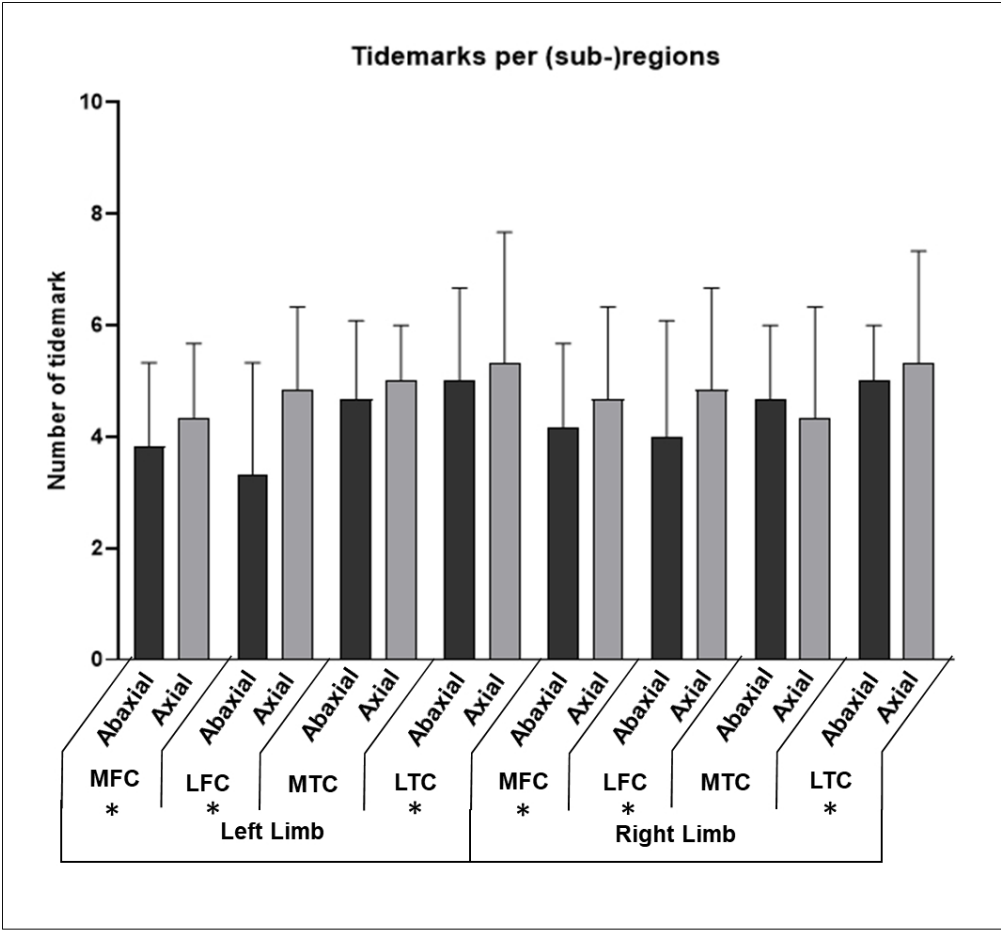


Figure 3: Number of tidemarks in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P<0.05$) is reached for the difference between the axial and the abaxial part of the region.
MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.

90x85mm (300 x 300 DPI)

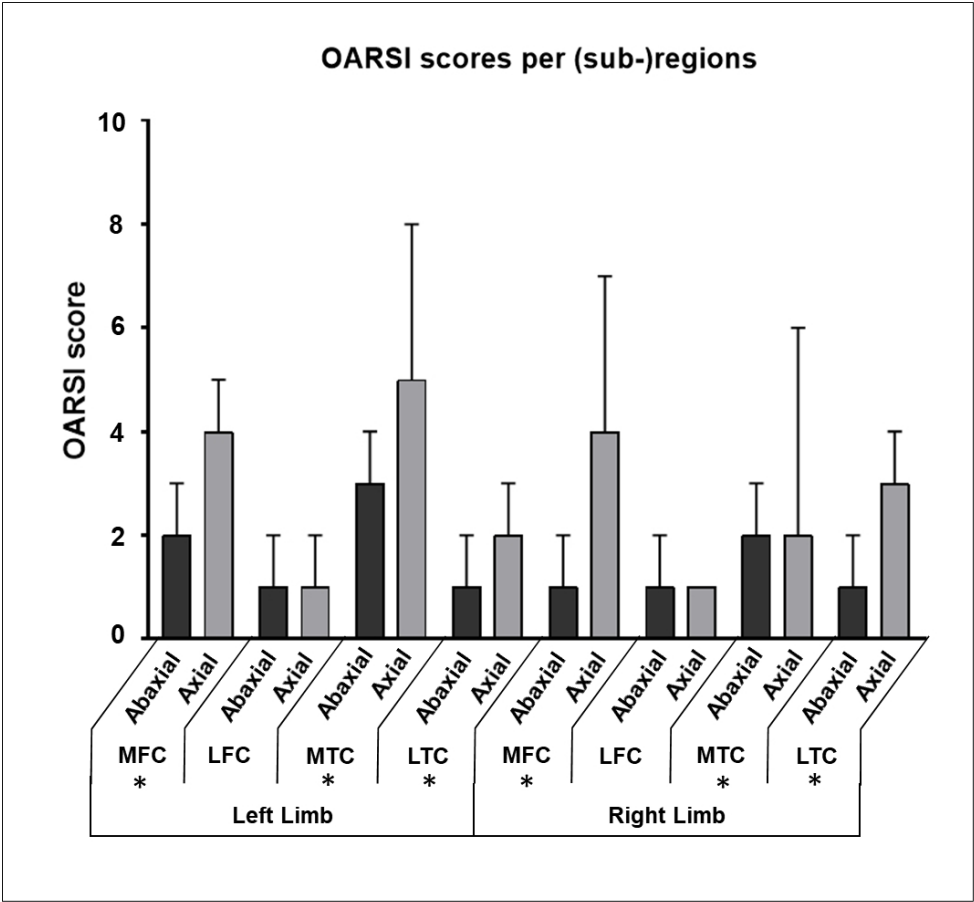


Figure 4: OARSI (OsteoArthritis Research Society International) scores in the different sub-regions for right and left limbs, expressed as median and interquartile range (bar). Asterisks means that statistical significance ($P<0.05$) is reached for the difference between the axial and the abaxial part of the region. MFC, LFC: medial and lateral femoral condyle, respectively; MTC, LTC: medial and lateral femoral condyle, respectively.

92x92mm (300 x 300 DPI)

Table 1: Tidemark count and OARSI score values (median and interquartile range) for the three age groups.

	6 months to 3 years old (N = 28)	4 to 6 years old (N = 31)	7 to 11 years old (N = 21)
Tidemark count			
Median	2.67	4.33	6.67
Range	(1.33 – 4.00)	(3.33 – 5.50)	(5.30 – 8.08)
OARSI Scores			
Median	1.5	2	3
Range	(1.00 – 3.00)	(1.00 – 5.00)	(1.00 – 7.00)

N= number of sheep. Mean tidemark count and OARSI scoring of both limbs were considered for each sheep.

The tidemark count ($P < 0.0001$) and the OARSI scores ($P = 0.0197$) differed significantly between groups.